

Role of Plants and Microbes in Bioremediation of Petroleum Hydrocarbons Contaminated Soils

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Received February 01, 2019; Revised March 17, 2019; Accepted March 25, 2019

Abstract Petroleum hydrocarbons contamination of soil, sediments and marine environment associated with the inadvertent discharges of petroleum-derived chemical wastes and petroleum hydrocarbons associated with spillage and other sources into the environment often pose harmful effects on human health and the natural environment, and have negative socio-economic impacts in the oil-producing host communities. In practice, plants and microbes have played a major role in microbial transformation and growth-linked mineralization of petroleum hydrocarbons in contaminated soils and/or sediments over the past years. Bioremediation strategies has been recognized as an environmental friendly and cost-effective alternative in comparison with the traditional physico-chemical approaches for the restoration and reclamation of contaminated sites. The success of any plant-based remediation strategy depends on the interaction of plants with rhizospheric microbial populations in the surrounding soil medium and the organic contaminant. Effective understanding of the fate and behaviour of organic contaminants in the soil can help determine the persistence of the contaminant in the terrestrial environment, promote the success of any bioremediation approach and help develop a high-level of risks mitigation strategies. In this review paper, we provide a clear insight into the role of plants and microbes in the microbial degradation of petroleum hydrocarbons in contaminated soil that have emerged from the growing body of bioremediation research and its applications in practice. In addition, plant-microbe interactions have been discussed with respect to biodegradation of petroleum hydrocarbons and these could provide a better understanding of some important factors necessary for development of *in situ* bioremediation strategies for risks mitigation in petroleum hydrocarbon-contaminated soil.

Keywords: plants, microbes, bioremediation, biodegradation, petroleum hydrocarbons, contaminated soils

Cite This Article: Aniefiok E. Ite, and Udo J. Ibok, "Role of Plants and Microbes in Bioremediation of Petroleum Hydrocarbons Contaminated Soils." *International Journal of Environmental Bioremediation & Biodegradation*, vol. 7, no. 1 (2019): 1-19. doi: 10.12691/ijebb-7-1-1.

1. Introduction

Petroleum hydrocarbons contamination of soil, sediments and marine environment is one of the significant global environmental hazards associated with exploration, drilling, development and production of crude oil and natural gas [1,2]. The inadvertent discharges of petroleum-derived chemical wastes and petroleum hydrocarbons associated with spillage into the environment, which is a major cause of controlled water and soil pollution in oil-producing regions, often pose harmful effects on human health and the natural environment, and have negative socio-economic impacts in the oil-producing host communities [1-9]. According to Ite et al. [1], petroleum (or crude oil) is a naturally occurring complex heterogeneous mixture containing mostly hydrocarbons and frequently contains significant amounts of nitrogen, oxygen and sulphur together with trace amounts of metals such as copper, iron, nickel, vanadium, and various elements. It is known that petroleum hydrocarbons occur naturally in great

abundance and in a variety of forms such as solid (e.g. asphalt), liquid (e.g. crude oil) and/or gaseous form (e.g. natural gas). Although petroleum industry has often classified the raw crude oil based on the geographic from which it was extracted, petroleum hydrocarbons can generally be divided into four main structural categories, viz. (i) the saturates, (ii) the aromatics, (iii) the asphaltenes, and (iv) the resins [2,6,10]. When petroleum hydrocarbons enter into the environment following accidental or operational discharges, soils and sediments are the ultimate sink for most petroleum hydrocarbon contaminants such as benzene, toluene, ethylbenzene, and xylenes (BTEX), aliphatic and polycyclic aromatic hydrocarbons (PAHs).

Petroleum hydrocarbons contamination of soils and sediment is a global environmental hazard and pose human health concern because of the refractory character of the aromatic components in the absence of oxygen [11] and the toxicity of some fractions as well as the tendency to contaminate food chains through bioaccumulation [2]. Depending on the geological features of the impacted site, microbiological and physicochemical parameters, some

organic contaminants within the contaminated sites may also undergo chemical and/or microbiological transformations that can influence fate and transport of many petroleum hydrocarbons environmental contaminants following spillage and/or inadvertent discharges. Although PAHs are widely distributed in nature and represent a major category of environmental organic chemical contaminants which make up about 5% by volume [12], aliphatic hydrocarbons are significant environmental organic contaminants of biogenic and anthropogenic sources in some parts of the world. It has been reported that predominant petroleum hydrocarbons pollution in the United Kingdom contains high volumes of aliphatic hydrocarbon fractions [13], while petroleum hydrocarbons pollution in the tropical region like the Nigeria's Niger Delta contains complex mixtures of both the aliphatic and aromatic hydrocarbon fractions [2,14,15]. However, microbial transformation and growth-linked mineralization by indigenous microbial communities play a major role in biodegradation of most petroleum-derived xenobiotic organic compounds such as benzene, toluene, ethylbenzene and xylenes (BTEX), aliphatic and polycyclic aromatic hydrocarbons [2,16].

Biodegradation is the general term used to describe the biological conversion, disintegration or transformation of organic contaminants by fungi, bacteria or other biological organisms to products that are generally lower in free energy [17]. Biodegradation involves either partial or complete mineralization of environmental organic contaminants by complex, genetically regulated physiological reactions catalyzed largely by microorganisms [18,19,20,21] and plants [22,23,24,25]. According to Leahy and Colwell [26], microbial degradation is often a growth-linked process that brings about mineralization and represents the primary mechanism through which petroleum hydrocarbon contaminants can be removed from the environment. Biodegradation of petroleum hydrocarbons in the environment has been well studied for decades and several factors that influence the rate and extents of petroleum hydrocarbons biodegradation (Table 1) have been reviewed by several researchers [2,16,26-45]. Compared to the expensive engineering techniques, biological degradation (biodegradation) strategies and/or bioremediation techniques are cost effective as well as environmental friendly approach for remediation of petroleum hydrocarbon-contaminated soils [2]. Apart from the actual chemical structure of the organic contaminants of concern, the suitability of a petroleum hydrocarbon-contaminated site for bioremediation can be affected by numerous abiotic factors. According to Ite and Semple [2], the successful application of biodegradation or biotransformation of organic contaminants in petroleum hydrocarbon-contaminated soils to less toxic end products often depends on the ability to establish and maintain favourable conditions that support microbial degradation both naturally and technologically. The interactions of plants with microbes in both soil and above ground shoot are of great importance for the growth and productivity of plants in agricultural and/or natural ecosystems as well as microbial degradation of petroleum hydrocarbons in soil. Plant-microbe interactions are considered to be important processes in the determination of the fate of organic

contaminants in the soil-plant ecosystem [46], the efficiency of microbial activity and microbial degradation or biotransformation of petroleum hydrocarbons in soil. In practice, effective understanding of the fate of an organic contaminant in the soil can help in the determination of the persistence of the contaminants in the terrestrial environment, promote the success of any remediation approach and help in the development of a high-level risk mitigation strategies.

In this review, we will provide a clear insight into the role of plants and microbes in microbial degradation of petroleum hydrocarbons in contaminated soil that have emerged from the growing body of bioremediation research and its applications in practice. Furthermore, plant-microbe interactions have been discussed with respect to biodegradation of petroleum hydrocarbons which could provide a better understanding of factors necessary for the development of *in situ* bioremediation strategies for risk mitigation in petroleum hydrocarbons contaminated soil.

Table 1. Linked Factors for optimum biodegradation of petroleum hydrocarbons in soil

| Factors | Optimum conditions |
|---|--|
| Microorganisms | Aerobic or anaerobic |
| Natural biological processes of microorganism | Catabolism and anabolism |
| pH | 5.5 – 8.8 |
| Temperature (°C) | 15 – 45 (mesophilic) |
| Soil type | Low clay or silt content |
| Soil moisture | 25 – 28 % of water holding capacity |
| Oxygen | Aerobic metabolism: > 0.2 mg/L dissolved oxygen and minimum air-filled pore space of 10% of the total volume |
| Nutrient | Carbon, nitrogen and phosphorus (C: N: P = 120:10:1 molar ratio) for microbial growth |
| Hydrocarbon concentration | 5 – 10 % soil dry weight |
| Redox potential | Aerobes and facultative anaerobes: Eh > 50 mV and anaerobes: Eh < 50 mV |
| Heavy metals | Total content 2,000 ppm |
| Microbial parameters | Adequate microbial cell count and metabolic activities |

Compiled from various sources [2,27,31,44,45,47,48].

2. Microbial Degradation of Petroleum Hydrocarbons

Petroleum hydrocarbons degrading microbes are ubiquitously distributed in the environment and biological degradation of petroleum hydrocarbons in the environment is ultimately one of the most important natural mechanisms through which these organic pollutants could be mineralized by indigenous soil microbes and/or transformed into harmless by-products. Over the years, several studies that focused on microbial degradation of petroleum hydrocarbons in the environment have been carried out to assess the fate of

n-alkanes [49,50], cycloalkanes [51], and aromatics [49,52,53,54,55,56]. Petroleum hydrocarbons differ in their susceptibility to microbial attack based on their molecular structures and generally differences in their susceptibility can be ranked as follows: *n*-alkanes > branched-chain alkanes > branched alkenes > low-molecular-weight *n*-alkyl aromatics > monoaromatics > cyclic alkanes > polynuclear aromatics > asphaltenes [2,27,57]. In the natural environment, various studies have shown that petroleum hydrocarbons are considered to be biodegraded mainly by a diverse group of bacteria, fungi and yeast (Table 2). Some petroleum hydrocarbon pollutants degrading microbes have the ability to biodegrade aliphatic hydrocarbons, some can biodegrade monoaromatic or polyaromatic hydrocarbons while various other hydrocarbon-degrading microbes can biodegrade resins [58]. Biological degradation of petroleum hydrocarbons under both anaerobic and aerobic condition has been clearly discussed by various researchers [59,60,61,62] and its pathways have been reviewed by some researchers [63-66]. In addition, biodegradation pathways for different petroleum hydrocarbons have been clearly reviewed by some researchers [2,67,68]. Over the past decades, several studies have adopted microbial degradation of organic contaminants as a sustainable approach for the clean-up of petroleum contaminated environments [2,27,47,69]. In practice, microbial degradation of petroleum hydrocarbon-chemical wastes and/or organic contaminants in soils are strictly limited by various factors [2,31,70]. The rates and extents of biodegradation and microbial growth in soil are influenced by a variety of abiotic factors, including the complexity and concentration of the organic contaminant mixtures, contaminants bioavailability and/or bioaccessibility, and organic contaminants interactions in soil, organic matter, temperature, pH, availability of nutrients (particularly nitrogen and phosphorus [N & P]), soil moisture level, availability of oxygen, concentration of organic contaminant of concern and redox potential [2,34-42,48,71].

Table 2. Predominant hydrocarbon-degrading microbes in the petroleum hydrocarbon-contaminated soil environment

| Bacteria | | | |
|---------------------------|-----------------------------|--------------------------|----------------------|
| Gram-negative bacteria | Gram-positive bacteria | Yeast | Fungi |
| <i>Pseudomonas</i> spp. | <i>Nocardia</i> spp. | <i>Aureobasidium</i> | <i>Trichoderma</i> |
| <i>Acinetobacter</i> spp. | <i>Mycobacterium</i> spp. | <i>Candida</i> | <i>Mortierella</i> |
| <i>Alcaligenes</i> sp. | <i>Corynebacterium</i> spp. | <i>Rhodotorula</i> spp. | <i>Penicillium</i> |
| <i>Flavobacterium</i> / | <i>Arthrobacter</i> spp. | <i>Sporobolomyces</i> | <i>Aspergillus</i> |
| <i>Cytophaga</i> group | | <i>Exophiala</i> | <i>Fusarium</i> spp. |
| <i>Xanthomonas</i> spp. | <i>Bacillus</i> spp. | <i>Trichosporon</i> spp. | |

Compiled from various sources: [27,31,35,67,68,72,73].

In a few review articles, Das and Chandran [27], Ite and Semple [2], Chandra et al. [48] and Srivastava et al. [71] have clearly discussed some of the most important factors that often affect the microbial degradation of petroleum hydrocarbons in the soil environment.

3. Plant-Microbes Interactions for Bioremediation of Petroleum Hydrocarbons Contaminated Soil

Biodegradation of petroleum hydrocarbons in soil can be limited by factors such as adsorption, mass transfer, bioavailability and bioaccessibility as well as the fate and behaviour of the hydrocarbon, while combined plant-microbial systems can lead to more efficient growth-linked mineralization of organic contaminant of concern in the root zone or rhizosphere [16,74]. It is known that plants often form associations with neighbouring plants, microflora, and micro-fauna, and most of these associations are facilitated by chemical signals exchanged between the host and the symbionts [75,76]. Although both detrimental and beneficial plant-microbe interactions are impacted by changes in the physical environment and ecosystem composition, utilization of the synergy between plants and rhizospheric microbes have a great potential to deal with petroleum hydrocarbons in effective soil remediation process. Plants and their associated rhizospheric microbes interact with each other and in most cases, plant often supplies the indigenous microbial population with a special carbon source that stimulates the microbial mineralization of organic contaminants by the indigenous microbial populations in the soil environment. Petroleum hydrocarbons present in the contaminated soil are degraded by plant-associated microbes which can involve endophytic and rhizospheric bacteria. Endophytic bacteria often colonize the internal tissue of nearly every plant and can promote plant growth, plant yield and also form a range of different useful relationships [77,78]. In addition, it is known that rhizospheric bacteria are a heterogeneous group of bacteria found in the rhizosphere, at root surfaces and in association with roots that can improve the extent or quality of plant growth directly and/or indirectly [79]. Finding from several studies have shown that endophytic bacteria often produce a wide range of natural products similar to some xenobiotics found in contaminated soil thereby enhancing microbial degradation of organic contaminants in soils (i.e., phytoremediation) [77,78,80]. There are several ways in which plants may increase the degradative potential of rhizospheric microbes, including general increases in microbial population densities, specific increases in petroleum hydrocarbon-degrading microbial communities, increased catabolic gene expression, increased horizontal transfer of catabolic genes, and enhanced bioavailability of hydrophobic hydrocarbons. Based on some researches that have been carried out over the years, it has been documented that plant secreted organic compound may enhance microbial degradation of petroleum hydrocarbons through various physical mechanisms such as availability of nutrients and organic contaminants transport, efficient attachments of microorganisms to host plants and aeration of impacted soils [81,82,83,84]. Although it has been reported in some studies that plants alone could be used for efficient biological remediation of petroleum hydrocarbon contaminated soils [85,86,87,88], the use of plants in associated with petroleum hydrocarbon-degrading microbes and/or plant growth-promoting bacteria (PGPB)

for the remediation of petroleum hydrocarbons contaminated soil has the advantage of reduction of the risks of residual contaminants and/or inverse transformation [80,89,90,91,92]. The use of phytoremediation strategies in conjunction with plant-associated microbes offers more potential for biological remediation than the use of a plant alone. During rhizoremediation (which involves combination of biostimulation and phytoremediation strategy), plant root secreted organic compounds stimulate the survival and microbial activity of petroleum hydrocarbon-degrading microbial communities and/or associated rhizospheric microbes, which subsequently result in effective microbial mineralization of organic compounds in impacted soil.

The plant root system, which was traditionally thought to provide anchorage and uptake of nutrients and water, is a chemical factory that mediates numerous underground interactions such as mutualistic associations with beneficial indigenous microbial populations, e.g. rhizobia, mycorrhizae, endophytes, and plant growth-promoting rhizobacteria (PGPR) [75]. Plant roots exude numerous compounds into the rhizosphere including sugars, amino acids, phenolics, and organic acids while the primary factor which impact the microbial degradation of petroleum hydrocarbons in rhizosphere is plant exudation. Organic chemical compound secreted by plant roots have traditionally been grouped into (i) low molecular weight compounds (LMWCs: simple sugar, amino acids, fatty acids, organic acids, phenolic compounds, aliphatic and/or aromatic compounds), and (ii) high molecular weight compounds (HMWCs: polysaccharides, polygalatic acids and proteins) [93,94,95]. Some of the organic acids exuded by plant roots include the citric acid cycle intermediates succinate, citric, malic, fumaric, oxalic and malonic acids are implicated in a variety of processes including microbial degradation of petroleum hydrocarbons in soil. Organic acids exuded from roots can alter the chemistry of the rhizosphere and subsequently alter the bioavailability of organic contaminants in soil [32]. This occurs either indirectly through promotion of the growth of indigenous petroleum hydrocarbon-degrading microbes or directly by changing conditions of the soil (e.g. pH) as well as alteration of the surface characteristics of soil. Root exudates can be mineralized by some soil microbial communities as growth substrates [96], and can further act as co-metabolites for the degradation of persistent organic pollutants [97,98,99,100]. It has been reported that the rhizospheric soil respiration is greater than that of the bulk soil, since CO₂ can originate not only from microbial respiration of soil organic C, but also from root respiration and microbial decomposition of rhizodeposition [101]. According to Ite et al. [32], plant roots secreted organic chemical compounds potentially supply indigenous microbial communities with micronutrients and the secretion of organic compounds from plant roots is an important process in the mediations of plant-microbe interactions. It has been reported that root exudates, including organic compounds which are analogues of polycyclic aromatic hydrocarbons (PAHs), may serve as nutrients sources for microbial growth and can stimulate the indigenous microbial degradation of organic contaminants in soil [102,103,104]. Few studies have shown that depending on their (bio)available concentrations and solubilities in soil, plant-derived

chemicals such as biogenic VOCs [105], hydroxycinnamic acids [33] and flavonoids [16] can either stimulate or inhibit microbial mineralization of organic contaminants. Evidence for the potential biological degradation of petroleum hydrocarbons through the rhizosphere effect has been provided, wherein plants exude organic compounds through their roots, influencing the abundance, diversity, or activity of potential rhizospheric petroleum hydrocarbon-degrading microbes [102]. Plants employ several complex mechanisms to restore soils contaminated with petroleum hydrocarbons [16,33] and plant-enhanced microbial degradation of organic contaminants in soil has been documented in several studies [102,106-112]. In a study, Ying et al. [112] reported that after four months plant cultivation, significant decreases of total petroleum hydrocarbon (TPH) concentrations were observed in the rhizospheres of *Scorzonera mongolica Maxim.*, *Atriplex centralasiatica*, and *Limonium bicolor*. Larger shoot and root biomass stimulated microbial biodegradation of TPH efficiently, higher average well colour development (AWCD) was observed in the rhizosphere of these three plants compared to the non-rhizosphere soil, which was strongly associated with the higher TPH degradation rate [112].

Soil indigenous microbial population often play significant roles in recycling of plant nutrients, soil structure maintenance, transformation of xenobiotics, controlling of plant pests using biological approaches as well as regulation of plant growth [113,114,115]. In pristine soil environments, petroleum hydrocarbon-degrading microbial population are often found at much lower numbers and the ubiquitous presence of some petroleum hydrocarbon fractions in soil has resulted in the maintenance of potential microbial activities of some soil microbial populations. The phylogenetic diversity of petroleum hydrocarbon degraders is enormous and several recurrent groups found in most phytoremediation studies are as follows: *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Flavobacterium*, *Mycobacterium*, *Micrococcus*, *Nocardioides*, *Pseudomonas*, *Ralstonia*, *Rhodococcus*, *Sphingomonas*, and *Stenotrophomonas* species in petroleum hydrocarbon-degrading strains [76,91]. In practice, the indigenous potential microbes can supplement the remediation aptitude of plants or diminish the phytotoxicity of the heavy metal-contaminated soil. Additionally, microbes and plants can form precise associations whereby the plant provides the microbes with alternative carbon source that stimulates the indigenous microbial populations to reduce the phytotoxicity of the contaminated soil [116]. According to Kumar et al. [116], plants and microbes can form nonspecific associations where common plant activities encourage the associated indigenous microbial community that results in the effective microbial metabolic activity to biodegrade contaminants in the soil. It is known that organic compounds secreted by plants root can alter soil chemistry by increasing ion solubility and availability. These biochemical processes often enhance microbial activities in the soil environment and subsequently improve the biological remediation of organic contaminants by indigenous microbes associated with plant roots [117]. Therefore, the variabilities and adaptabilities of plant and microbial degradation are very important in the reduction

of the risks associated with petroleum hydrocarbon pollutants in the soil environment. Soil microbial communities degrade petroleum hydrocarbons through various different catabolic pathways and plants may affect the degradability potential of soil microbial communities [2]. In practice, effective application of plant–microbe interactions for the bioremediation of petroleum hydrocarbons contaminated soil depends primarily on the presence of indigenous microbial populations (plant associated rhizospheric and endophytic bacteria) with specific genes required for petroleum hydrocarbons biodegradation [76]. Several researches have demonstrated that interactions between plants and microbes are very important for the biotransformation and/or microbial transformation of organic contaminants in the soil environment [33,74,118,119,120,121]. Overall, plant–microbe interactions have contributed to remediation of petroleum hydrocarbons contaminated soil through a series of metabolic transformations, biological, chemical and physical processes.

4. Biological Remediation Strategies and Their Applications

Bioremediation is a process whereby biological degradation processes are utilized to eliminate, attenuate or transform organic contaminants and pollutants to mainly carbon dioxide, water, and biomass in order to mitigate risks. Bioremediation functions through exploitation of the diverse metabolic capabilities of microbes to detoxify or remove organic contaminants. Over the years, biodegradation and/or bioremediation has become the preferred the remediation strategy for the clean-up of petroleum hydrocarbon–contaminated soil given that the method is inexpensive and environmentally sustainable, and can accelerate naturally occurring biodegradation processes via the optimisation of limiting factors [2,45,122]. It is widely known that bioremediation technologies use the catabolic capacity of both plants and microbes to decontaminate or reduce the concentration of environmental hazardous contaminants in soil to levels of risk that are acceptable to site owners and/or regulatory agencies [24,25,123,124,125,126,127]. The choice of the most effective bioremediation technologies among various range of remediation strategies developed to treat organic xenobiotics depends on three basic principles – contaminants bioavailability, contaminants bioaccessibility contaminant and the unrestraint of optimization of biological activity. Biological remediation strategies can be categorized into microbial remediation (involving microbial communities) and phyto–/rhizo–remediation (involving plant and associated rhizospheric microbial communities). It is known that microbial remediation strategies might be enhanced (e.g. bioaugmentation, biosparging, bioventing, phytoremediation and rhizoremediation), while other strategies might be carried out without any form of enhancement (e.g. intrinsic bioremediation or natural attenuation). In practice, microbial remediation strategies for contaminated medium (e.g. soil and water) may be performed *in situ* (on site) or *ex situ* (the contaminated medium may be excavated or pumped out of the original location) under aerobic or

anaerobic conditions. Although biological degradation of xenobiotic organic compounds can occur under both aerobic and under anaerobic conditions, the aerobic bioremediation strategies (which can be classified as *ex situ* and *in situ*) are most effective for the treatment of petroleum hydrocarbon–contaminated soil. It has been reported that microbial remediation and phytoremediation are the two main bioremediation strategies effective for the treatment of petroleum hydrocarbon–contaminated soil [128]. In practice, there are several factors that affect the choice of bioremediation strategies for treatment of petroleum hydrocarbon–contaminated soil. Some of these sites specific factors include type and nature of contaminant, mobility, concentration and volume of contaminant, bioavailability and mobility of the contaminant, structure and texture of the contaminated soil, geology of the impacted area, the proximity to structures and potential receptors, and intended end use after clean-up [2,129,130].

Bioremediation strategies for petroleum hydrocarbons contaminated soil could be divided into *ex situ* and *in situ* approaches. *Ex situ* bioremediation techniques are biological processes for decontamination that involve the removal of contaminated material from its original position and its treatment either on-site or at another location (e.g. biopiling, bioreactors, composting, and land farming) [2,45,131]. On the other hand, *in situ* bioremediation techniques are biological processes for decontamination that are performed with the contaminated material (soil and groundwater) left in its natural or original position (e.g. bioaugmentation, bioventing, biosparging, biostimulation, monitored natural attenuation, windrow, phytoremediation and rhizoremediation) [45,131]. According to Das and Adholeya [47], *in situ* bioremediation techniques are predominantly the most cost effective approaches for treatment of contaminated material and fewer disturbances are involved since biological processes for decontamination are carried out on site avoiding excavation and transport of contaminants. The optimization, control of biological transformations and/or microbial transformations of xenobiotic organic contaminants in an environment, which is a complex system affected by several factors, require the adoption of multidisciplinary approaches towards effective bioremediation strategies development. Some of the *in-situ* bioremediation techniques are clearly discussed below.

5. Biostimulation

Biostimulation is one of the most environmental friendly methods of microbial degradation of petroleum hydrocarbons that involves the modification of the contaminated environment to stimulate metabolic activities of indigenous microbial communities capable of mineralizing organic contaminants. The use of biostimulation to decontaminate petroleum hydrocarbons in contaminated soils has been developed based on biological processes that support aerobic condition, nutrients availability and optimum moisture in order to enhance indigenous microbial activity and subsequent microbial degradation [13,132–135]. According to Hazen [135], biostimulation is dependent on the indigenous

microbial communities which requires that they be present and that the contaminated environment be capable of being altered in a way that will have the desired bioremediation effect and/or targeted goal. Over the years, numerous advantages of biostimulation strategies in the remediation and/or treatment of petroleum hydrocarbon-contaminated sites have documented in several researches around the world [136-147]. Based on published literature and investigations by different researchers around the world, there seem to be inconsistencies in biostimulation studies and inorganic amendments and/or fertilizers are the most commonly used nutrients with high biodegradation efficiency [132,144,148,149,150,151]. Few studies investigating the effect of different types of biodegradation strategies on the coastal region around Prince Sound in Alaska contaminated by Exxon Valdez oil spill, revealed that the addition of fertilizers – composed mainly of nitrogen and phosphorus – accelerated removal of oil approximately 5 times more rapidly [152,153]. The effect of different types of inorganic amendments and/or fertilizers and different delivery strategies in a low-energy, sandy beach or in a salt marsh have been investigated and documented in several studies over the years [154-158]. Findings from these studies showed that biostimulation through periodic amendment with inorganic fertilizers (e.g. ammonium nitrate and triple super phosphate) increased the rate of petroleum hydrocarbon degradation on beaches [154-158].

The effectiveness of biostimulation of petroleum hydrocarbon-contaminated soils have been intensively studied and documented in several researches over the past three decades. In a study, Bento et al. [141] reported that amendment of contaminated soil with biosolids is a more effective and efficient biostimulation strategy compared to the commonly approach of inorganic fertilizer amendment. The effectiveness and efficiency of the strategy is attributed to biosolids amendment abilities to supplement carbon in petroleum hydrocarbon-contaminated soils. In a related field study, Lee *et al.* [157] carried out comparative performance of inorganic fertilizers with organic fish bone-meal fertilizer and the results obtained showed that the organic fertilizer had the greatest effect on microbial growth and activity, while the inorganic fertilizers were most effective in microbial degradation of petroleum hydrocarbons. Although traditional application techniques have been found to have negative impacts on soil microbes in few studies [36,139], addition of inorganic fertilizers and/or urea has been reported to have no effect on microbial degradation of petroleum hydrocarbon-contaminated soil [139,141,159,160]. In a similar study, Ayotamuno et al. [132] reported that the amendment of a polluted agricultural soil with NPK (nitrogen, phosphorous, and potassium) fertilizer significantly enhanced the rate of crude oil biodegradation and the findings showed that the initial concentration of 84 mg kg⁻¹ TPH was reduced by 50 – 95 % in the test cells. In another study, Andreolli et al. [142] carried out biostimulation of contaminated soil with a microbial growth promoting formulation amendment and findings from the biostimulation strategy resulted in an abatement of 70 % for C₁₂₋₄₀ and 100 % for PAHs compounds within 60 days. It has been reported in a related study that biostimulation resulted in 60 % petroleum hydrocarbon

degradation and biostimulation with nitrogen and phosphorus enhanced microbial growth and activity [143].

The amendment of petroleum hydrocarbon-contaminated soils with organic and inorganic fertilizers are important strategies for bioremediation and the application of these amendments often requires scientific and technological knowledge [161]. Apart from inorganic fertilizers, many studies have confirmed the validity of biostimulation of soil microbial activity through addition of organic fertilizer and/or organic wastes [147,161-167]. In a biostimulation study, Agamuthu et al. [165] investigated potential use of organic wastes in the enhancement of the microbial degradation of waste lubricating oil in contaminated soil and the results obtained showed that cow dung and sewage sludge can be an effective organic amendment for the microbial degradation of waste lubricant-contaminated soil. Nwankwegu et al. [167] investigated the application of inorganic fertilizer (NPK) and organic manure (compost) amendments in the bioremediation of diesel-contaminated agricultural soil and the results obtained showed that contaminated soil amended with organic manure resulted in > 90 % removal of total petroleum hydrocarbons within two months. In addition, the findings from the phytotoxicity test further showed that organic manure was most effective and efficient amendment in the bioremediation of the petroleum hydrocarbon-contaminated soil. In a similar study, Adesodun and Mbagwu [147] investigated the use of some organic wastes from animal droppings as amendments to biostimulate microbial degradation of waste lubricating oil (spent oil) in artificially contaminated soil. The results obtained showed that the total hydrocarbon content (THC) was significantly reduced following amendment with cow dung (CD), poultry manure (PM) and pig wastes (PW). Adesodun and Mbagwu [147] reported that PW amendments resulted in the highest net percentage loss in THC for soils contaminated with 5000 mg kg⁻¹ (0.5 %) and 50,000 mg kg⁻¹ (5 %) spent oil levels during the first (1st) year. Addition of poultry manure stimulated the highest reduction in soils spiked with medium oil concentration, i.e. 2.5 % spent oil (25,000 mg kg⁻¹). The overall net loss of THC stimulated by each organic waste in the second (2nd) year showed that PM addition was more effective and efficient amendment irrespective of total spent oil loading. According to Adesodun and Mbagwu [147], PW addition was effective at low level of oil contamination, while PM addition was more effective at high level of oil contamination. The overall order of the performance and/or efficiency differences between these organic waste amendments are as follows: PM > PW > CD. In another study, Agarry et al. [164] investigated the application of animal manure and chemical fertilizer as amendments during four (4) weeks *in situ* remediation of petroleum hydrocarbon mixture (kerosene, diesel oil, and gasoline mixtures) (10 % w/w) contaminated soil. From the results obtained in this study, Agarry et al. [164] reported that petroleum hydrocarbon-contaminated soil amended with poultry manure, piggery manure, goat manure, and NPK fertilizer resulted in 73 %, 63 %, 50 %, and 39 % total petroleum hydrocarbon degradation, respectively. In practice, from the complex nature of both inorganic and organic nutrients amendments during remediation of

petroleum hydrocarbon-contaminated soil, biostimulation should be combined with other strategies in attempts to developed environmentally friendly and cost effective bioremediation strategies.

6. Bioaugmentation

Bioaugmentation is the practice of introduction of either indigenous or exogenous catabolically active microbial communities to petroleum hydrocarbon-contaminated soil when metabolic activity is low [2]. It is known that the introduction of a single strain or a known mixed microbial consortium with desired metabolic and catalytic capabilities often accelerate the microbial degradation of sites contaminated with petroleum hydrocarbons. In practice, consultation of the local regulations is very important step when considering bioaugmentation in order to decide what type of biota could serve as the best option for bioremediation [168]. According to Forsyth et al. [169], some of the factors to be considered when adopting bioaugmentation of contaminated soils are as follows: (i) soils with non-detectable number or inadequate numbers of xenobiotic organic contaminant-degrading microbial populations; (ii) soils containing contaminant mixtures that require multiple treatment or remediation processes, including processes that could have adverse effects or toxic to microbial populations, and (iii) for contaminated sites for which the cost of bioaugmentation strategy is cheaper than the cost of other available alternative methods. It is apparent that successful utilization of bioaugmentation strategy requires not only the knowledge of type and level of contaminants but also suitable strains of microbes or consortia of microbes with the effective catabolic potential to mediate petroleum hydrocarbons degradation [169-171]. According to Vidali [45], the two factors that limit the application of microbial cultures in remediation of contaminated soil are: (a) nonindigenous microbial consortium rarely compete well enough with an indigenous microbial population to develop and sustain useful microbial population levels and (b) most soils with long-term exposure to petroleum hydrocarbons have indigenous microbial communities with effective biodegradative capabilities if the contaminated soil is well managed. Over the years, the application of bioaugmentation as a potential bioremediation strategy for remediation of petroleum hydrocarbon-contaminated soils has been reported in several studies around the world [141,142,143,145,168,170,172-189]. In another study, Bento et al. [141] reported up to a four-fold increase in the microbial activity of bioaugmented diesel-contaminated soils with a corresponding higher degradation extents (75.20 ± 0.20 %) of light petroleum hydrocarbon fraction ($C_{12} - C_{23}$) in the bioaugmented treatment condition compared with the degradation extent of 48.7 ± 0.3 % observed in the naturally attenuated treatment condition. For heavy petroleum hydrocarbon fraction ($C_{23} - C_{40}$), degradation extent of 45.70 ± 0.40 % was reported for the biostimulated condition compared to degradation extent of 72.70 ± 0.40 % obtained in the bioaugmented condition using Long Beach soil, which initial concentrations of TPH contamination were 2800 mg kg^{-1} ($C_{12} - C_{23}$) and 9450 mg kg^{-1} ($C_{23} - C_{40}$). In a related study, Nwankwe

and Onwosi [184] evaluated two bioaugmentation forms for the degradation of total petroleum hydrocarbon (TPH) in gasoline contaminated soil, microbial activity indexed by dehydrogenase (DHA) assay and effect of pH on biodegradation were also measured during the 8 week incubation. From the results obtained, the highest percentage of degradation (75.70 %) was observed in *Micrococcus luteus*, 71.10 % in *Rhizopus arrhizus* and 66.40 % in the consortium though removal efficiencies were not statistically different for the bioaugmentation options but significantly different ($p < 0.05$) compared to the percentage of degradation in the control [184]. In another bioaugmentation study, Gargouri et al. [187] reported significant higher petroleum hydrocarbons removal efficiencies in the augmented treatment condition compared to that obtained in the control condition and the results obtained showed the total petroleum hydrocarbon reduction of 63.4 mg g^{-1} to 2.5 mg g^{-1} at the end of the treatment.

Bioaugmentation, which is a relatively new concept in wastewater treatment process using biological reactors, is frequently utilized in treatment of municipal wastewater, industrial wastewater, agricultural waste wastewater and petroleum hydrocarbon-contaminated medium (e.g. soil and ground water). In a study, Ayotamuno et al. [173] reported that the total hydrocarbon content (THC) reduction in oily sludge ($69,372 \text{ mg kg}^{-1}$ THC) varied between 40.70 % – 53.20 % within 2 weeks as well as between 63.70 % – 84.50 % within 6 weeks of application of the catabolic microbial inoculum. The colony forming units (CFUs) of the treatment condition amended with bio-preparation varied between 1.2×10^{12} and 3.0×10^{12} CFU/g of sludge and decreased to 7.0×10^{11} CFU g⁻¹ of sludge at the end of the six (6) week of applying the bioremediation. A comparison of the performance of the indigenous microbial populations in the control sample, the added bio-preparation showed accelerated rate of reduction of THC in the oily sludge [173]. In a related study, Odjadjare et al. [182] investigated the relationship between growth profile and the extent of biodegradation, and the results obtained showed that the specific growth rates of axenic cultures of the bacteria during degradation of Escravos light crude oil ranged between 0.0037 and 0.0505 h^{-1} , while that of the mixed cultures varied from 0.0144 to 0.1301 h^{-1} . The results further showed that for single cultures, crude oil biodegradation ranged from 28.71 – 99.01 % and from 12.38 – 91.58 % for the mixed cultures [182]. In a similar study, Oboh et al. [177] investigated the relevance of 15 petroleum hydrocarbon-degrading bacterial and fungal isolates and the predominant species isolated primarily belonged to the genera *Pseudomonas* and *Aspergillus*. The results obtained showed maximal increase in optical densities and total viable counts concomitant with a decrease in pH of the culture media. According to Oboh et al. [177], generation times usually varied between 0.64 d and 1.09 d, 0.97 d and 3.03 d, 0.88 d and 2.97 d for kerosene, diesel and naphthalene, respectively. The bacterial and fungal isolates mineralized petroleum hydrocarbons as sole carbon and energy sources with no statistical difference ($P > 0.05$) in the rates of mineralization, which further suggest close genetic similarities for each isolates in terms of biodegradation capabilities [177]. Similarly, Okoh [179]

investigated the rates of petroleum hydrocarbon degradation of different strains of *Pseudomonas aeruginosa* and the results showed evidence of significant reductions (between 6.50 % and 70.60 %) of major peak components of the petroleum hydrocarbons. George-Okafor *et al.* [175] investigated the petroleum hydrocarbon degradation potential of indigenous fungal isolates from petroleum hydrocarbon-contaminated soils and found that two isolates (*A. versicolor* and *A. niger*) exhibited > 98 % degradation efficiency for PAHs when grown in a culture medium containing 1 % crude oil and 0.1 % Tween 80 for 7 days. In practice, the most effective and efficient bioaugmentation strategy could be achieved by utilizing microbial inoculants isolated from petroleum hydrocarbon-contaminated site that have aged for several decades [2,170]. Over the years, some researchers have suggested that mixed microbial cultures with broad enzymatic capacities are necessary for the treatment of sites contaminated with complex petroleum hydrocarbon mixtures [190,191,192]. According to Ite and Semple [2], effective understanding of the individual roles played by each consortium member is very important in influencing the efficacy of microbial consortium and their exploitation in the successful implementation of bioaugmentation strategies for the treatment of petroleum hydrocarbon-contaminated sites. In practice, the choice of best microbial culture for bioaugmentation strategy should take into consideration the following features: fast growth, easily cultured, ability to withstand high concentrations of organic contaminants and the ability to survive in a wide range of environmental conditions [169,170,171].

In a study, Andreolli *et al.* [142] carried out a comparative assessment of bioaugmentation (inoculation with a suspension of *Trichoderma sp. mycelium*) and biostimulation (addition of a microbial growth promoting formulation) as strategies for the bioremediation of a burned woodland soil contaminated with environmental xenobiotic organic compounds. From the results obtained, it was observed that the best biodegradation efficiency for high molecular weight (HMW) hydrocarbons was reached 60 days after soil treatment through the biostimulation protocol and about 70 % of the initial concentration of HMW hydrocarbons was removed. However, about 55 % biodegradation was obtained with the bioaugmentation protocol within the same period, while 45 % biodegradation was obtained with the natural attenuation [142]. In a recent study, Al-Kharusi *et al.* [186] investigated the effect of bacterial quorum sensing (QS) signals on the respiration activity of an petroleum-contaminated soil with and without the addition of an alkane-degrading bacterial consortium. From the results obtained, addition of acyl homoserine lactones (AHLs) to contaminated soils increases respiration activities and degradation rates, while the respiration activities were affected by the concentration of AHLs [186]. According to Al-Kharusi *et al.* [186], the addition of AHLs has a stimulating effect on bacterial respiration activities and degradation of petroleum hydrocarbons, hence it can be useful in bioaugmentation treatments of crude oil-contaminated soils. Over the years, several strategies and approaches have been developed to augment the catabolic potential at petroleum hydrocarbon-contaminated site (especially in soils with low number of indigenous

hydrocarbons-degrading microbes) and enhance the biodegradation of recalcitrant fractions. In some cases, some of these bioremediation strategies involve the utilization of genetically engineered microorganisms and gene bioaugmentation. The success of bioaugmentation strategy strongly depends on the ability of microbial inoculants to survive in petroleum hydrocarbon-contaminated soil. The efficiency of bioaugmentation is determined by many biotic factors (quick growth, easy to cultivate, tolerance to high concentrations of xenobiotic organic contaminants, competition between indigenous and exogenous microbial communities for carbon sources) and abiotic factors (concentration and availability of contaminant, availability of nutrients, type and chemical structure of organic contaminants, temperature, humidity, pH, organic matter, aeration, soil type and physico-chemical properties of soil, contaminant, chemical composition of the root exudates, etc.). Development of effective and efficient bioaugmentation strategy may be achieved by delivering suitable petroleum hydrocarbon-degrading microbial communities immobilized on various carriers or use of activated soil as well as optimization of other related factors for optimum biodegradation.

7. Phytoremediation

Phytoremediation (the direct use of living green plants for *in situ* remediation) refers to the use of plant-based processes and their associated microbial communities (in a symbiotic interaction) to remove, transform, stabilize and/or mineralize inorganic and organic contaminants in soil, sludges, sediment, surface water and groundwater [144,193-201]. Phytoremediation is a biological treatment process that utilizes physical, chemical, and biological processes to remove, degrade, transform, or stabilize contaminants in soil and groundwater [199,202]. There are various mechanisms involved in phytoremediation of petroleum hydrocarbon-contaminated soil and some of these mechanisms include the following:

- Phytoextraction: is cost effective plant-based technology for remediation of contaminated land and it is also known as phytoabsorption, phytoaccumulation, or phytosequestration. Its mechanism involves the process whereby plants remove petroleum hydrocarbons from soil and concentrate them in the harvestable above ground portion of the parts of the plants [188,200].
- Phytodegradation: this is also known as phytotransformation and its mechanism involves the degradation of organic contaminants into simpler compounds directly, through the release of enzymes from roots, or through metabolic activities within plant tissues and the process sometimes support plant growth [200,203].
- Phytofiltration: is a promising environmentally friendly technology that involve the use of plants (both terrestrial and aquatic), plants roots (rhizofiltration) or seedlings (blastofiltration) and their associated rhizospheric microbial communities to remove, absorb, concentrate, precipitate and/or sequester organic xenobiotic compounds in contaminated environment. According to Lee [204],

phytofiltration involves filtration of water and soil through a mass of tissues to remove xenobiotic compounds or nutrients in the contaminated medium.

- **Phytovolatilization:** this mechanism involves the absorption and/or uptake of xenobiotic organic compounds by plant roots and its conversion to a gaseous state, and subsequent release and/or transpiration into the atmosphere in the form of volatile contaminants [200,205,206]. It is known that phytovolatilization of xenobiotic organic compounds in soil could occur either directly or indirectly as clearly reviewed by Limmer and Burken [206] to differentiate between direct- and indirect-phytovolatilization.
- **Phytostimulation:** this is also known as rhizodegradation and its mechanism involves a situation whereby roots release organic compounds in order to enhance microbial and catabolic activities in the rhizosphere through the rhizospheric associations between plants and symbiotic indigenous soil microbial communities [207,208].
- **Phytostabilization,** which is also known as phytoimmobilization, aims to contain xenobiotic organic compounds within the vadose zone through absorption by roots, adsorption onto root surface or precipitation within the area of plant roots or rhizosphere [200]. It is known that this mechanism involves a situation whereby plants reduce mobility of contaminants by preventing off-site contamination through their migration and bioavailability of petroleum hydrocarbons in the environment by immobilization [209].
- **Phytohydraulics:** its mechanism involves a situation whereby plants are used to increase evapotranspiration, thereby controlling soil water and contaminant movement [210].

It is well known that each of the above mechanisms often have a significant impact on the volume, environmental fate and behaviour, toxicity, bioaccessibility and bioavailability of petroleum hydrocarbons in contaminated soil. In practice, various plants can stimulate environmental xenobiotic organic compounds loss/removal by accumulation and transformation [211], by extracellular transformation [212,213] and by stimulation of microbial metabolic and catabolic activities within the area of plant roots [207,208]. Apart from agricultural environment monitoring, plants can be used as environmental friendly and sustainable bioremediation tool to mitigate risks associated with a wide range of environmental xenobiotic compounds such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl's (PCBs), pesticides, petroleum hydrocarbons, and heavy metal [116]. Phytoremediation, which is an efficient and environmentally friendly biotechnological tool for the clean-up of contaminated sites, involve the use of plant and their associated microbes' interactions have been reviewed by some researchers over the years [214,215,216,217]. Phytoremediation strategies for xenobiotic organic compounds can be categorized into two classes, viz., direct phytoremediation (*in planta*) and phytoremediation *ex planta* [102,218,219]. The

phytoremediation *ex planta* is based on a synergistic relationship between root exudates (organic substances that are secreted plant roots into the rhizosphere zone) and metabolic activities of indigenous rhizospheric associated microbial communities [111]. Phytoremediation can be inexpensive for large petroleum hydrocarbon-contaminated sites with low concentrations of residual hydrocarbon fractions, sufficient nutrient, or metal pollutants, where contamination does not pose significant threat to any ecological receptor [220,221].

Plants with petroleum hydrocarbons phytoremediation ability have been documented in several studies as shown in Table 3. According to Eapen and D'Souza [222], a plant effective for phytoremediation strategy should possess the following characteristics: (i) ability to tolerate, accumulate, or degrade pollutants in their aboveground parts, (ii) tolerance to pollutants concentration accumulated, (iii) fast growth and high biomass, (iv) fibrous root systems, and (v) easy harvestability. The fibrous root systems provide a larger surface area than taproots for microbial populations colonization [102] and also facilitate interaction between the indigenous rhizospheric associated microbial communities and the xenobiotic compounds [223]. Apart from the laboratory-based experiments and/or studies, phytoremediation has been tested successfully in the field to clean up petroleum hydrocarbon-contaminated soil over the past 20 years. In a study, Njoku *et al.* [224] investigated plants that could be used in the enhancement of bioremediation of petroleum hydrocarbon-contaminated soil and the results obtained showed that the growth of *Glycine max* significantly affected the pH, moisture and organic matter contents of petroleum hydrocarbon-contaminated soils at different levels of significance ($P < 0.001$, $P < 0.01$ and $P < 0.05$). In a study, Njoku *et al.* [224] reported that biodegradation of petroleum hydrocarbons was enhanced in soil contaminated with 25 g crude oil in the presence of *G. max* and the soils became more favourable for plant growth as weeds sprouted from the petroleum hydrocarbon-contaminated soil in the presence of *G. Max*. Based on the findings of the study, the cultivation of some plants, such as *G. max*, could be effective and efficient in the risks mitigation and clean-up of petroleum hydrocarbon-contaminated soil. Phytoremediation is cost effective plant-based bioremediation strategy for the clean-up of petroleum hydrocarbons contaminated soils especially in the tropical region with insufficient financial resources [225,226,227,228]. Over the years, some studies have been carried out to demonstrate enhanced microbial degradation of xenobiotic organic compounds in planted soil in comparison to unplanted soil [148,229-232]. To date, a small number of studies have carried out on biostimulation with phytoremediation strategy in the clean-up of petroleum hydrocarbon-contaminated soils.

In a few studies, biostimulation strategies were combined with phytoremediation to enhance microbial degradation of petroleum hydrocarbons in soil [148,233]. According to Ayotamuno *et al.* [233], these biostimulation studies were mainly cases of phytoremediation where fertilizers were applied to support plant growth, and both bioremediation strategies were applied simultaneously. Ecological rehabilitation, which is the practice of restoring contaminated or degraded ecosystems, with the cultivation

of Vetiver grass (*Vetiveria zizanioides*) has been found to significantly increased biomass and subsequently enhance the phytoremediation of an oil shale mined land contaminated with heavy metals [234]. Vetiver grass, a xerophyte and a hydrophyte, is highly tolerant to various abiotic stresses and it has long been used to rehabilitate coal and gold mining and mining overburdens [235,236,237]. In another related study, Lu *et al.* [232] found that goose grass (*Eleusine indica*) significantly enhanced phytoremediation of soil contaminated with TPH and PAHs.

Table 3. Plants with Phytoremediation Ability for the Clean-up of Petroleum Hydrocarbon-contaminated Soil

| S/N | Scientific Name | Common Name |
|-----|--|------------------------|
| 1 | <i>Agropyron smithii</i> | Western wheat grass |
| 2 | <i>Andropogon geradi</i> | Big bluestem |
| 3 | <i>Bassia scoparia</i> L. | Burningbush or ragweed |
| 4 | <i>Biden</i> | Beggar ticks |
| 5 | <i>Bouteloua gracilis</i> | Blue grama |
| 6 | <i>Bouteloua curtipendula</i> | Side oats grama |
| 7 | <i>Buchloe dactyloides</i> | Common buffalo grass |
| 8 | <i>Chloris gayana</i> | Bell rhode grass |
| 9 | <i>Cynodon dactylon</i> | Bermuda grass |
| 10 | <i>Daucus carota</i> | Carrot |
| 11 | <i>Elymus canadensis</i> | Canada wild rye |
| 12 | <i>Fetusca rubra</i> var. <i>arctared</i> | Arctared red fescue |
| 13 | <i>Glycine max</i> | Soybean |
| 14 | <i>Lemna gibba</i> | Duckweed |
| 15 | <i>Lolium multiflorum</i> | Annual ryegrass |
| 16 | <i>Lolium perenne</i> L. | Ryegrass |
| 17 | <i>Medicago sativa</i> L. | Alfalfa |
| 18 | <i>Oryza sativa</i> or <i>Oryza glaberrima</i> | Rice |
| 19 | <i>Panicum virgatum</i> | Switch grass |
| 20 | <i>Panicum coloratum</i> | Verde klein grass |
| 21 | <i>Phaseolus vulgaris</i> L. | Bush bean |
| 22 | <i>Populus deltoids nigra</i> | Poplar tree |
| 23 | <i>Pueraria montana</i> var. <i>lobata</i> (Willd.) Maesen | Kudzu |
| 24 | <i>Secale cereal</i> L. | Winter rye |
| 25 | <i>Schizachyrium scoparius</i> | Little bluestem |
| 26 | <i>Sorghastrum nutans</i> | Indian grass |
| 27 | <i>Sorghum bicolor</i> | Sorghum |
| 28 | <i>Sorghum vulgare</i> L. | Sudan grass |
| 29 | <i>Zea mays</i> L. | Maize |
| 30 | <i>Zoysia japonica</i> var. <i>meyer</i> | Meyer zoysia grass |

Adapted from various sources [198,238-243].

Compared to trees and shrubs, herbaceous plants, especially grasses show characteristics of rapid growth, large biomass, strong resistance to the contaminated environment, and effective stabilization to contaminated soils and in efficient rehabilitation of contaminated lands in the tropical and subtropical regions [233,234,244]. The potential of common tropical grasses, such as elephant grass (*Pennisetum purpureum*), to enhance the decontamination of a crude oil contaminated soil has been reported [233]. In practice, phytoremediation can be applied at sites moderately contaminated with petroleum hydrocarbons or after the application of other bioremediation strategies to further mitigate risks associated with residual petroleum hydrocarbons in soil

[238]. In order to further optimize this bioremediation strategy, phytoremediation efficiency of plants may be substantially improved using genetic engineering technologies [245].

The use of phytoremediation strategies in the enhanced biodegradation of xenobiotic organic compounds within the rhizosphere zone of some selected cultivars (plants) have been documented over the past years [246,247,248]. It has been demonstrated in some studies that the availability of root exudates often enhance microbial degradation of petroleum hydrocarbons for: (i) plant roots grown in contaminated soils [249,250]; (ii) root exudates sampled from plant roots and applied to xenobiotic compounds contaminated soils [106,247], and (iii) addition of artificial root exudate mixtures to contaminated soils [251]. By flushing sterile exudates direct from the roots of corn (*Zea mays* L.) into attached soil columns, Yoshitomi and Shann [247] demonstrated that root exudates were responsible for enhanced microbial mineralization of pyrene. In the study, Yoshitomi and Shann [247] suggested that plant root exudates might have stimulated the enhanced biodegradation of polycyclic aromatic hydrocarbons (PAHs) in the rhizosphere. In another study, Miya and Firestone [106] found that the amendment of contaminated soil with root exudates collected from slender oat (*Avena barbata* Pott ex Link) maintained higher populations of petroleum hydrocarbon-degrading microbes and increased the biodegradation of phenanthrene. In another study, the degradation rates of 3 to 5 ring polyaromatic hydrocarbons increased with the addition of mineral nutrients and an artificial root exudate mixture [251]. In a related study, Phillips *et al.* [110] discovered that root exudates from wildrye (*Elymus angustus* Trin.) and alfalfa (*Medicago sativa* L.) resulted initially in lower mineralization rates for naphthalene, phenanthrene and *n*-hexadecane compared to an unplanted control. The positive effect of root exudates, phenolic compounds, terpenes and flavonoids on microbial activity and microbial degradation of polychlorinated biphenyls (PCBs) in soil have been demonstrated in some studies [252,253,254]. Over the years, several studies have demonstrated the use of plants in enhanced microbial degradation of some xenobiotic organic compounds in soil [102,106-111]. The use of plants in petroleum hydrocarbons degradation via rhizosphere effect has been demonstrated and evidence suggested that organic compounds secreted by plants roots often influence microbial abundance, diversity, and/or catabolic activity of indigenous rhizospheric microbial populations [102].

Root exudation is one of the most important factors that often affect performance of indigenous rhizospheric associated microbial populations and the potential driver that stimulated enhanced microbial degradation of petroleum hydrocarbon-contaminated soil. There are numerous possible mechanisms by which root exudates may enhance microbial degradation of xenobiotic organic compounds in the rhizosphere. Some of these mechanisms include direct mineralization of petroleum hydrocarbons via the action of plant derived enzymes, enhancement of contaminant bioavailability, activation of microbial enzymatic pathways and/or provision of an energy/nutrient source to the microbial community [81]. Plant roots secrete various organic compounds

(phytochemicals) into the rhizosphere where these secreted organic compounds take part in multi-partite interactions and subsequently alter biochemical and physical properties of the rhizosphere [255,256,257,258]. According to Martin *et al.* [81], the presence of carboxylates in the root exudate could enhance biodegradation of petroleum hydrocarbons by: (i) provision of an alternative energy source; (ii) increase availability of phosphorus, and/or (iii) promotion of bioavailability of organic contaminants in soil. Plant secreted organic chemical compounds such as salicylic acid, monoterpenes and flavonoids, may enhance microbial and biological transformation of xenobiotic organic compounds with similar structures [118]. In addition, plant secreted organic chemical compounds could also induce genes encoding enzymes involved in the degradation process, promote bioavailability of contaminants, and/or increase the microbial catabolic activity and enrichment in the number of petroleum hydrocarbon-degrading microbial communities [74,119,120]. In a study, Narasimhan *et al.* [259] investigated the enhancement of plant-microbe interactions using a rhizosphere metabolomics approach and observed that a large number of aromatic acids or phenylpropanoids, including flavonoids, in plant exudates can be applied in bioremediation of contaminated soils with pre-existing vegetation. Flavonoids, which are common to tracheophytes and also higher plants other than legumes [260], are known components of plant root exudates [261], plant tissue and plant based products. The plant root systems can promote the movement of microbial communities through soil and penetration of impermeable soil layers as the roots grow [91], which subsequently result in efficient microbial degradation of petroleum hydrocarbon-contaminated soil. The emission of volatile organic compounds (VOCs) from soil, either by roots or by decomposing biomass, and subsequent consumption within the rhizosphere form a significant part of the carbon cycle [262-264] and the presence of VOCs in soil could enhance biodegradation of xenobiotic organic compounds [265,266,267]. Although soils may act as sources or sinks of VOCs, depending on the (bio)available concentrations and solubilities in soil, plant-derived organic chemicals such as biogenic VOCs [105], hydroxycinnamic acids [33] and flavonoids [16] can either stimulate or inhibit microbial mineralization of xenobiotic organic compounds. Although phytoremediation strategies has been extensively investigated over the years, there is insufficient information on specific mechanisms and the complex role of plant-secreted organic compounds on biodegradation of xenobiotic organic compounds in soil [32].

8. Rhizoremediation

Rhizoremediation is a process whereby microbes degrade xenobiotic organic compounds in the rhizosphere and this phytoremediation strategy involves the use of plants and their associated interactions with the rhizospheric microbes. Rhizoremediation is one of the most effective phytoremediation technologies used for the removal of organic contaminants with the most active region for the remediation of soil contaminants being near

the roots of the plants. According to Anderson *et al.* [102], the utilization of the rhizospheric microbial communities and plants in the phytoremediation of petroleum hydrocarbon-contaminated soil is referred to as rhizoremediation. In practice, associated rhizospheric microbial communities are the main contributors to the microbial degradation process and the living plant in this phytoremediation strategy can be viewed as biological, solar-driven pump and treatment systems [268,269,270]. It is known that the living plant can extract, accumulate, degrade, volatilize or vaporize soluble xenobiotic organic compounds from the contaminated environment through water and mineral uptake, transport, partitioning, translocation, assimilation and transpiration systems [268]. Rhizoremediation is a specific form of phytoremediation strategy that involves the use of plants in association with their associated rhizospheric microbial communities and bioaugmentation with specific microbial communities (contaminant degraders and/or plant growth-promoting bacteria [PGPB]) could be adopted for optimization of these techniques [87,91]. It is known that rhizoremediation (the use of plant and microbial communities' interaction) is the eco-friendly and cost-effective bioremediation strategy that involves the removal of xenobiotic contaminants from waste products of contaminated sites by mutual interaction of plant roots and suitable microbial population [91,271]. Rhizoremediation has been proven to be cost-effective and efficient for a wide range of organic contaminants including various petroleum hydrocarbons [271-275] as well as polychlorinated biphenyls (PCBs) [276,277]. According to Cook and Hesterberg [271], trees and grasses are often used for phytoremediation, with trees typically chosen for the bioremediation of BTEX while grasses are usually used for the bioremediation of petroleum hydrocarbon-contaminated soil. Wojtera-Kwiczor *et al.* [275] investigated the rhizoremediation of diesel-contaminated soil with two rapeseed varieties and the petroleum hydrocarbon-degrading microbial communities and the results obtained revealed different responses of the plant defense mechanisms. In another study, Meng *et al.* [273] found that the average remaining percentage of PAHs in mixtures (48 %) was significantly lower than those in monocultures (55 %) and nonplanted soils (70 %). Overall, the results obtained from this study showed that plant-promoted biodegradation accounted for almost 99 % plant-enhanced PAH losses and plant uptake only contributed less than 2 % of PAH reduction in the contaminated soil [273]. According to Meng *et al.* [273], phyto-/rhizo-remediation is dependent on plant species and plant-promoted biodegradation is the most important pathway for removal of PAHs during phytoremediation of petroleum hydrocarbon-contaminated soil.

Apart from other limitations of the technique, the success and efficiency of plant species utilization during rhizoremediation might depend on: (i) fibrous root systems to harbour large numbers of microbial communities; (ii) ability to promote primary and secondary catabolic activities, and (iii) establishment of survival and ecological interactions with indigenous microbial communities [278]. In practice, the effective and successful utilization of rhizoremediation strategy in field mainly depend on the capacity of organic

contaminant degrading–microbial communities and/or plant growth promoting bacteria to efficiently colonize the rhizosphere [278,279]. It is known that the success of beneficial processes is based on the competence of the rhizospheric associated microbes, which is reflected by the ability of the microbes to survive in the rhizosphere, compete for the exudate nutrients, to be sustained in sufficient numbers, and efficiently colonize the growing root system [278,280]. The use of both plants and microbes in biological degradation of petroleum hydrocarbons has proved to be a cost-effective approach and further improvement can be achieved by the application of specifically tailored genetically modified (GM) plants/microbes and use of optimum conditions to ensure effective remediation potential of organic contaminants. Plant–assisted bioremediation (rhizoremediation) stands out to be a potential tool for effective microbial degradation of petroleum hydrocarbons contaminated soil and adequate combination of plant species and microbes could enhance the clean-up process.

9. Conclusions

Plants and microbes have played a major role in bioremediation of petroleum hydrocarbons contaminated soils over the years and bioremediation has been recognized as an alternative to traditional physico-chemical approaches to restore contaminated sites. The findings from both laboratory and field scale studies have demonstrated that many microbial communities possess the inherent ability to transform or utilize organic contaminants (e.g. petroleum hydrocarbons) as carbon and energy sources. In order to successfully remediate petroleum hydrocarbon–contaminated soils, several biochemical and physical factors that affect biodegradation should be properly controlled to optimize the environmental conditions for the contaminant–degrading microbial communities. The success of biodegradation strategy for petroleum hydrocarbon–contaminated soil depends on significant environmental conditions, the chemical structure of the pollutants, contaminant bioavailability, the presence of catabolically active microbes; and the organic pollutant–matrix interactions [2,37,42,71,281,282]. Although there are many *ex situ* and *in situ* bioremediation strategies for the treatment of petroleum hydrocarbon–contaminated soil, it is necessary to have a deeper understanding of the microbial ecology of contaminated sites so that bioremediation strategies could be improved since each contaminated site will be different. The knowledge of the fate and behaviour of petroleum hydrocarbons in an impacted soil can help determine the persistence and degradability of the organic contaminant in the environment and subsequently, the success of any remediation method. A better understanding of plant–microbe interactions could be exploited to further develop phytotechnologies for site clean-up and application of the bioremediation strategies for petroleum hydrocarbons contaminated soil through a series of metabolic transformations, biological, chemical and physical processes.

Acknowledgements

The authors would like to thank the Research and Development Coordinator of Akwa Ibom State University, Nigeria and Engr. Emem E. Ite of Infiniti Innovative Group – USA for providing all the support for successfully completing this review paper.

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